Growth responses of Atriplex lentiformis and Medicago arborea in three soil types treated with saline water irrigation.

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Growth responses of *Atriplex lentiformis* and *Medicago arborea* in three soil types treated with saline water irrigation

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Abstract

Large amounts of industrial wastewater, often of high in salt content, are produced by urban activities or industries which require reuse or disposal and thus can be potentially used in agriculture to ease the pressure on freshwater supply for irrigation. At the same time, plant performance in the field may be determined not only by salt concentration *per se* but also by confounding effects associated with soil physical and chemical properties. The purpose of this study was to investigate the effect of saline water irrigation 90 to 16 dS/m range) on the performance of two plant species, *Atriplex lentiformis* (xero halophyte) and *Medicago arborea* (glycophyte) grown in three soil texture setups – (1) clay, (2) sandy loam and (3) sandy loam over clay (texture-contrast) - under glasshouse conditions. Both plant species yielded higher biomass in the clay texture compared to other soil texture setups under all irrigation treatments. There was no significant variation in chlorophyll fluorescence with salt treatments but stomatal conductance was significantly reduced (up to 70%) by salinity in *M. arborea*. Overall, leaf ion content (Na^+ and Cl^-) also increased with increasing salinity treatment in both plants, but significant effects were seen only in sandy loam soil for both species. Both osmotic effect and specific ionic toxicity impacted physiological performance in *M. arborea* while *A. lentiformis* plants were insensitive to both components of salt stress. Plant performance in the sandy soil was not as good as in clay, indicating that soil texture and structure may have a significant role in the salt stress process under saline irrigation.

Key words: *Atriplex lentiformis*; *Medicago arborea*; saline irrigation; soil types; osmotic effect; physiological performance

1. Introduction

Global food production is strongly related to the available land area and water resources and other input resources available. Clearly it needs to match the burgeoning human population without degrading the resources used to produce it. The world population has increased from 5.7 billion in 1994 to 7.2 billion in 2014 and it is projected to reach 8.1 billion in 2025 and 9.6 billion in 2050 (United Nations, 2014). This increase will increase food demand by as much as 70% by 2050 (Bruinsma, 2009). At the global level, 70% of all available freshwater is already used for irrigated agriculture, and in under-developed countries this exceeds 90% (FAO, 2014). This indicates that freshwater resources are already overstretched and cannot sustain demand from the irrigated agriculture without major increases in efficiency. Due to the high demand and competition for freshwater, from both urban and industrial users, alternative sources of irrigation water are needed along with selected plant species and increases in water use efficiency. There are various sources of low quality water, i.e. saline water, that can be used for irrigation purpose, such as water coming from natural or secondary salinized aquifers (Ayers and Schoneman, 2006), subsurface drainage from irrigated fields (Grattan et al., 2004), industrials sources (Gerhart et al., 2006) and brine from desalinisation plants (Jordan et al., 2009). However, most conventional crops cannot tolerate very saline environments and their production under these conditions may be economically unsustainable. It has been estimated that global salt induced land degradation and resulting production losses in irrigated areas could be as high as US$27.3 billion per year (Qadir et al., 2014). One alternative is to use saline waters and land for the production of halophytic crops. There is an increasing number of halophytes which have been tested and used for food, fodder, fuel production purposes and landscaping purpose (Panta et al., 2014; Ventura et al., 2015; Pessarakali, 2015).

Atriplex lentiformis is a native widespread halophytic shrub of south-western united States and northern Mexico and it has been used for various purposes such as forages, re-vegetation and rangeland enhancement programs around the world (Brownings et al., 2006) and as use as an irrigated forage (Watson et al., 1987; Glenn et al., 2009; Jordan et al., 2009). The productivity of *A. lentiformis*, however, can vary dramatically depending on irrigation regimes and soil types and range between 1 and 20 t/ha. For example, in USA *A. lentiformis* produced 12 to 20 tonnes dry matter per hectare per year under saline water irrigation (Watson et al., 1987). Similar numbers were reported for a field trial in the Coastal Sonort

Desert (Glenn and O'Leary, 1985). Research conducted in Pakistan showed that *A. lentiformis* produced up to 8 tonnes dry matter/ha/year under rainfed condition (Mahmood et al., 1993). However, in Western Australia, the productivity of the *Atriplex* plantations was very low and did not exceed 1 t/ ha /year in saline texture-contrast soils under rainfed conditions (Barrett-Lennard et al., 1990). So the causal link between soil types, irrigation schedule and concentration of salt in the irrigation water has to be established to optimize *Atriplex* production as water is important factors for the productivity of many *Atriplex* species in arid regions (Belkheiri and Mulas, 2013a). As a check species we have used *Medicago arborea*, a salt-tolerant glycophyte that is adapted very well to infertile and rocky soil and shows a high potential as a valuable fodder for low rainfall climatic conditions with alkaline soil (Dear et al., 2003). *M. arborea* is popular for its high nutritive values and preference given by small ruminants (Amato et al., 2004). However, a concentration of 100 mM NaCl significantly reduced the plant growth in *M. arborea* (Sibole et al., 2003). It remains to be shown to what extent *M. arborea* can be used for saline agriculture purposes, and how its production is affected by soil types and amounts of irrigation water.

High irrigation rates can cause waterlogging problems in clayey textured soils, and it is estimated that 10 % of global land is adversely affected by waterlogging (Setter and Waters, 2003). The major constraints are depleted oxygen and associated hypoxia leading to internal energy stresses (Bailey-Serres and Voesenek, 2008) and inability to maintain membrane potential (Shabala et al., 2014). Waterlogging also causes reduction in soil redox potential, and increased production of toxic compounds by plant roots (Shabala, 2011; Zeng et al., 2013). As a result of these changes, plant stomatal conductance and leaf water potentials will decrease and plant shoot and root growth will reduce and ultimately the plant may die (Barrett-Lennard, 2003). The combination of salinity and waterlogging stress is highly detrimental to plant growth and is usually more severe than each stress per se (Barrett-Lennard and Shabala, 2013). Thus, the optimal choice of saline irrigation regime was expected to be affected not only by the amount of dissolved salts but also by the soil drainage property and plant's ability to deal with constraints imposed by soil flooding.

Most previous studies have been conducted with only a single soil texture or irrigation regime. Given the speculation of possible difference in plant performance based on the growing condition, it was necessary to compare plant responses to salinity in a range of different soil textures and irrigation regimes. Therefore, the aim of this work was to compare the phenotypic and physiological responses of *Atriplex lentiformis* and *Medicago arborea* to

different levels of irrigation salinity in clay, sandy loam and texture-contrast (sandy loam/clay) soil under two irrigation levels.

2. Material and methods

2.1 Plant material and experimental setup:

Atriplex lentiformis and *Medicago arborea* seeds were obtained from the AustraHort Seed Merchant, Queensland. Seedlings were grown in a glasshouse at the Horticultural Research Centre at the University of Tasmania in Hobart, Australia in 2012. Two-month old equal sized seedlings of *A. lentiformis* and *M. arborea* were transplanted into 30 cm tall five litres plastic pots having one of the following: (1) sandy loam, (2) clay soil and (3) texture-contrast (sandy loam/clay) soil. The nutrient properties of this soil are presented in Table 1. A nappy liner was put at the base of each pot to prevent the loss of soil materials during drainage. The texture-contrast soil profile was artificially made by filling the lower half of the pot with clay soil and the upper half with sandy loam soil. After 3 weeks of transplanting, salinity treatments were started. Plants were irrigated with three levels of saline water (0.04 dS/m, 8 dS/m, 16 dS/m) prepared by adding appropriate amount of NaCl to the tap water. Irrigation was given at the rate of 700 mm/year and 1400 mm/year rate (hereafter, it is described as 'low' and 'high' irrigation rate respectively). Irrigation water was applied twice a day via drip irrigation system. Total water applied per pot/day was 340 ml in low irrigation and 680 ml in high irrigation regimes. The use of 700 mm/year and 1400 mm/year saline water was to coincide with the field experiment irrigation rate and with the aim of using as much as saline water that plant can handle in an acceptable way. The pots were arranged in a randomized complete block design on the bench inside the glass house with five replicates per species. After treatment initiation, plants were allowed to grow for five months and then harvested for biomass measurement.

2.2 Photosynthetic and gas exchange parameters:

Measurements of stomatal conductance were carried out on sunny days (with light intensity of ca 1100 µmol/m^2/s) from the adaxial surface (upper surface) of the youngest fully developed leaf under glasshouse conditions. The measurements were taken in well watered plants by using a leaf porometer (model SC-1, Decagon Devices, Inc. USA) between 9 to 11

am and 1 to 3 pm. After that chlorophyll content in the leaf was measured by chlorophyll meter SPAD 520 (Konika, Minolta, Sensing, Inc. Japan). The maximum photochemical efficiency of photosystem II (PSII; chlorophyll fluorescence Fv/Fm ratio) was recorded from the upper surface of the fully developed leaves by using a hand held chlorophyll fluorometer (OS-30P, Opti-Science, Inc. USA) in dark adapted plants as described in Smethurst and Shabala (2003).

2.3 Osmolality and leaf ion content:

The youngest fully-expanded leaf was harvested and placed into Eppendorf tubes and stored in a freezer. The frozen leaf was defrosted and the sap was extracted by hand-squeezing the leaf samples as described in Cuin et al. (2009). The extracted leaf sap was centrifuged at 2000 RPM for 10 min to remove solid residues. Approximately 20 µl of the supernatant was used to measure sap osmolality with a vapour pressure osmometer (Vapro; WescorInc, Logan, UT, USA). An additional 50 µl of the collected supernatant was diluted with 5 ml of distilled water and used for the determination of Na^+ and K^+ concentration (in mM) using a flame photometer (Corning 410C, Essex, UK). Five replicates for each treatment for both plant species were assessed. The Cl^- was quantified from the remaining diluted supernatant by Cl^- selective microelectrodes using commercially available Cl^- liquid ion exchanger (catalogue No. 24902, Sigma-Aldrich, St. Luis, MO, USA) using MIFE system as described elsewhere (Shabala et al., 2006). Electrodes were calibrated in a set of three standards encompassing measured ranges of Cl^- ions using a three-point calibration (ranging from 0.5 to 180 mM). Electrodes with a Nernst slope of less than 50 mV per decade and/or correlation less than 0.999 were discarded from measurements. Calibrated chloride sensitive microelectrodes were placed into a beaker containing a sample solution and voltage values were recorded by the MIFE CHART software (Shabala et al., 2006). The samples were measured for several minutes until a steady reading of ion flux were observed on the computer screen. The recorded values in mV were converted into concentration values using the calibrated Nernst slope of the electrode using the MIFEFLUX software (Shabala et al., 2006). The data was stored in ASCII format on a personal computer and transferred to Excel for data analysis.

2.4 Biomass:

At harvest, plant leaves and stems were separated and fresh weights were measured. Plants were dried at 70 °C for 72 h and dry weights were recorded.

2.5 Data analysis:

Data were analysed by analysis of variance using Proc GLM in SAS 9.2 for the overall test and means. Pairwise differences were compared using the least significant difference method at the 5% level of probability. The statistical significance of correlations between data sets was calculated using Pearson's r values. Different low-case letters in each panel of the figures indicate significance at $p < 0.05$.

3. Results

3.1 Leaf biomass (fresh weight and dry weight)

Both the amount of salt in the irrigation water and soil type had a significant impact on plant performance. Salinity levels up to 16 dS/m did have a strong positive influence on plant performance in *A. lentiformi*s (Suppl. Fig. 1) but there was a negative effect for *M. arborea* (Suppl. Fig. 1). Plant fresh leaf weight showed, on average, a positive effect of quantity and quality of irrigation water on the growth of *Atriplex lentiformis* in all treatments. The fresh leaf biomass yield of *Atriplex lentiformis* varied by 3.5 times among the treatments, ranging from 24 g/plant to 84 g/plant (Fig. 1). The yield increased significantly ($p<0.05$) when the irrigation salinity levels increased from 0.04 ds/m to 8 dS/m. The t-test (LSD) shows a significant effect ($p<0.001$) of soil types and salt levels on the fresh leaf biomass yield. The mean fresh leaf yield of plants grown in sandy loam, texture-contrast and clay soil were 45.5 g, 54.1 g, 75.5 g, respectively (differences significant at $p<0.05$; data not shown).

Thus, salinity level up to 16 dS/m appeared to be beneficial for the growth of *Atriplex lentiformis.* The leaf fresh weight (LFW) and leaf dry weight (LDW; Fig 2 A and B) yield varied significantly ($p<0.001$) based on the soil types and water salinity levels but not to the irrigation level. The yields (LFW and LDW) were higher in clay soil compared to sandy loam soil. The mean LDW yield of the plant grown in sandy, duplex (texture-contrast) and clay soil were 10.0 g, 10.4 g and 16.8 g/ plant respectively.

In *M. arborea*, the LFW and LDW were significantly decreased in the texture-contrast and sandy loam soil at 16 dS/m compared to the clay soil. Interestingly, both LFW and LDW were higher in clay soil at 8 dS/m salinity from both low and high irrigation levels in all salinity treatments (Fig. 1 and 2). Overall, soil types and salinity levels show a significant ($p<0.001$) effect on leaf yield.

3.2 Quantum yield of Photosystem II (PSII)

The maximum quantum yields of PSII (chlorophyll fluorescence Fv/Fm values) were above 0.72 and 0.78 in both *A. lentiformis* and *M. arborea* respectively (Suppl. Fig. 2), and there was no significant difference between salinity treatments. If comparison were made based on soil textured PSII performance was always higher in the sandy loam soil for *A. lentiformis* but same trend was not observed in *M. arborea*.

3.3 Stomatal conductance (*gs*)

There was no significant impact of salinity on stomatal conductance in *A. lentiformis* regardless the salt concentrations, soil types and irrigation levels with overall average stomatal conductance values being around 25-30 mmol $m^{-2}s^{1}$. The stomatal conductance was always lower in sandy soil compared to other soil types in the low irrigation treatment (significant at $p< 0.05$); however, it was the clay soil which had the lower values in high irrigation treatment.

A significant reduction in stomatal conductance at 16 dS/m was observed in *M. arborea* (Fig. 3). It is also noted that conductance was higher in the clay soil in both 8 and 16 dS/m treatments compared to other the soil texture situations. In *M. arborea,* reductions in stomatal conductance at 8 and 16 dS/m salinity levels were 41% and 74% of control respectively. The effect of irrigation levels on stomatal conductance was not significant in *Medicago arborea* ($p>0.05$).

3.4 Leaf chlorophyll content (SPAD measurements)

The leaf chlorophyll content is an important parameter in determining the photosynthetic rate and a sensitive indicator of plant stress. In this study, salinity had significant ($p=0.001$)

impact on chlorophyll content in *A. lentiformis* but quantity of the applied water did not show such effect. It was also observed that leaf chlorophyll values were higher (non-significant) in the sandy loam compared to the other soil textures in high irrigation in 8 and 16 dS/m treatments but the same trends were observed in clay soil in low irrigation treatments (Fig. 4).

In *M. arborea,* leaf chlorophyll values were significantly affected by soil texture ($p = 0.0017$) and salt levels ($p = 0.0025$). The highest leaf chlorophyll value was observed in clay soil at 8 dS/m salinity in both high and low irrigations. If comparison is made within the other soil texture, the average leaf chlorophyll value was lower in sandy loam soil in both 8 and 16 dS/m water salinity. In addition, leaf chlorophyll values decreased with increasing salinity treatments in the texture-contrast and sandy soil textures in both high and low irrigation.

3.5 Ion concentrations

The leaf sap Na^+ increased 1.5 – 2 fold compared with control plants in *A. lentiformis*, whereas in *M. arborea* 6 and 9 fold increments were reported for plants grown at 8 and 16 dS/m salinity levels, respectively (Fig. 5). In both species, leaf Na^+ concentration was higher in the sandy loam and lowest in the clay textured soil (significant at $p<0.05$). For *A. lentiformis* and *M. arborea* Na^+ concentration was significantly ($p<0.05$) higher with increased salinity treatment but no effect occurred between irrigation rates.

A decline in leaf K^+ concentration was observed when salinity levels increased in both plant species (Fig. 6). The reduction was 40 and 60% in *A. lentiformis* and 50 to 60 % in *M. arborea* of control at 8 and 16 dS/m salinity treatments, respectively. Plants grown in clay soil had comparatively higher K^+ compared to the sandy loam and texture-contrast soils for both species. However, the irrigation levels had no significant effect on the leaf K^+. A comparison between the two species show *A. lentiformis* had 2 – 3 folds more K^+ than *M. arborea*.

Leaf Cl^- accumulation was significantly increased in both species with increasing salinity treatments (Fig. 7). Average leaf Cl^- levels were 111, 439 and 636 mM in *Atriplex lentiformis* and 40, 193 and 448 mM in *Medicago arborea* at 0.04 , 8 and 16 dS/m salinity treatments, respectively (all significant at $p< 0.001$). It shows that salt-treated *A. lentiformis* had accumulated 4 and 6 times more Cl^- in its leaves compared to control, while in *M. arborea* Cl^- was 5 and 11 times more when grown at 8 and 16 dS/m salinity than in the

control treatment, respectively. At 16 dS/m higher Cl^- was observed in *A. lentiformis* in the clay soil but for *M. arborea* it was higher in the texture-contrast and sandy loam textures. In general the higher Cl^- concentrations were observed in *A. lentirormis* than in *M. arborea* but the rate of irrigation had no significant effect on Cl^- accumulation in the plant leaf.

3.6 Leaf Osmolality

Leaf sap osmolality significantly increased in salt treated plants in both species and the trend was similar in both soil types and irrigation levels (Fig. 8). At the same time, a strong positive correlation ($p<0.001$) was observed between Na^+, Cl^- and osmolality (Table 2). In addition, there was a negative correlation between leaf Na^+ and chlorophyll concentration (Suppl. Fig. 3) in *M. arborea* ($R^2 = 0.32$) and *A. lentiformis* ($R^2 = 0.17$). Similarly, leaf Na^+ was also negatively correlated with the stomatal conductance in *M. arborea* ($R^2 = 0.41$) but not in *A. lentiformis* ($R^2 = 0.003$).

4. Discussion

Differential growth responses between plant species under saline condition

Atriplex lentiformis and *Medicago arborea* plants irrigated with saline water showed drastically different biomass responses, with *Atriplex* plants benefiting from the applied salt and *M. arborea* growth becoming more stunted as the level of salt increased in the irrigation water (Suppl. Fig. 1). The reduction in the biomass of *M. arborea* with increased levels of salt concentration appears to be a consequence of both osmotic stress and specific ionic toxicity caused by the applied saline water. The supporting evidence for the first component comes from stomatal conductance (*gs*) data. Decreased stomatal conductance limits net photosynthesis and diversion of energy to produce compatible solutes in saline environment (Flowers et al., 2015). This conclusion is supported by the marked declining *gs* value observed in *M arborea* at high salinity (Fig. 3) which is expected to reduce net CO_2 assimilation (Munns, 2002) and led to decreased growth rate (Suppl. Fig. 1). The physiological rationale behind this reduction could be an attempt to decrease the water loss under the conditions of "physiological drought" imposed by salinity (Shabala, 2013). The closure of stomata or reduction of stomatal conductance is related to both increased ABA production under saline conditions (Ashraf and Harris, 2013; Durner, 2013) and reduced K^+ availability to maintain turgor pressure in guard cells (Anschutz et al., 2014). This effect also causes in reduction of photosynthesis with decreased diffusion of CO_2 to chloroplast (Netondo et al., 2004). Salt-treated wheat plants also show a positive relationship between stomatal conductance and relative growth rate, where higher CO_2 assimilation rate was a result of higher stomatal conductance (James et al., 2008). In this work, significantly reduced stomatal conductance in high salinity treatments in *M. arborea* indicates that plants suffered from osmotic stress. The effect was observed in reduction in leaf biomass yield (Fig. 1). However, in *A. lentiformis* similar trend were not observed suggesting it is equipped with highly efficient mechanisms to ensure efficient control of stomata (hence, sustained CO_2 assimilation) under saline conditions.

In many salt-affected plants species the reduction of stomatal conductance at high salinity treatments results from both an inability of the plant to maintain water balance (Sutka et al., 2011; Shavrukov, 2013) and a reduction in the root hydraulic conductivity (Calvo- Polanco et al., 2014). The closing of stomata could be a defence mechanism of plants to avoid excessive

water loss (Yang et al., 2005). Reduced stomatal conductance in salt-affected plants may also be associated with a down-regulation of PIP aquaporin gene expression (Boursiac et al., 2005). However, in *A. lentiformis* stomatal conductance was not significantly decreased, as in *M. arborea*, when plants were exposed in salinity treatments and indeed it was stable. It would therefore be interesting to examine the level of expression and the activity of aquaporins in *Atriplex* roots, and compare these with *M. arborea* plants.

Due to the similarity in physical and chemical properties between Na^+ and K^+, the former competes with the later for the binding sites thus affecting a large number of metabolic enzymes in the cytosol (Anschutz et al., 2014). This also impacts the cell elongation rate. This was not the case for *A. lentiformis* which displayed a typical halophytic response with suboptimal growth in the no salt and enhanced growth under mild to moderate salinity stress as found in previous research (Short and Colmer, 1999). The positive correlation ($R^2 = 0.41$, $p<0.01$) between *A. lentiformis* leaf biomass and leaf Na^+ concentration (Table 2) suggests that Na^+ was used by *A. lentiformis* to maintain plant growth, most likely as a major osmolyte used for turgor maintenance and cell expansion. However, this correlation was negative ($R^2 = -0.49$, $p<0.01$) for *M. arborea* indicating inability of this species to utilise Na^+.

Another possible aspect worth discussing is the presence of highly dense and multilayered epidermal bladder cells in *Atriplex* species (Shabala, 2013). Traditionally, the role of these bladder cells has been attributed to external sequestration of excess Na^+, taking it away from the metabolically important mesophyll cells. As the diameter of epidermal bladder cells is often approx. 10-fold bigger than epidermal cell, they could sequester 1000-fold more Na^+ compared with epidermal cells (Shabala and Mackay, 2011). However, these bladders may have been playing an important role as a secondary epidermis to reduce water loss when exposed to saline environment (Adams et al., 1998). In addition to this, some reports suggest that halophyte species may substitute K^+ for Na^+ to increase turgor pressure rapidly in stomatal guard cell (Shabala and Mackay, 2011) which can be justified by the higher concentration of K^+ in *A. lentiformis* compared to the *M. arborea* (Fig. 8).

Plant growth can also be adversely affected by both high cytosolic Na^+ or Cl^- concentrations and low cytoplasmic K^+ (Smethurst et al., 2008; Shabala, 2009; Demidchik et al., 2010; Flowers et al., 2015, Nieves-Cordones et al., 2016). The low K^+ concentration at 8 and 16 dS/m salinity in both plants indicates that either there was a restriction of the K^+ uptake, or

poor K^+ retention in plant tissues (Fig. 6). The K^+ retention ability of plants has been linked to the salinity tolerance in some plant species (Chen et al., 2008; Smethurst et al., 2008). A reduced cytosolic K^+ concentration can negatively affect cell metabolism (Shabala and Cuin, 2008) and cause programmed cell death due to the activity of caspase-like proteolytic and endonucleolytic enzymes in the salt affected plants (Shabala, 2009; Demidchik et al., 2010,). However, the reduction of K^+ concentration in *A. lentiformis* was not below the optimum level of cytosolic K^+ (100 – 150 mM) needed for protein synthesis in plant (Walker et al., 1998). On the other hand, reduction of K^+ concentration in *M. arborea* was below this threshold. So this may be one of the reasons for reduction of *M. arborea* growth in saline treatments while such impact was not noticeable in *A. lentiformis* under same conditions.

The chlorophyll fluorescence (Fv/Fm) data provided further insights into the physiological responses of these plants to salinity treatments. Our results reveal that there was no significant reduction in Fv/Fm values in both plant species when grown in up to 16 dS/m salinity (approximately 160 mM). Similar to the earlier suggestions (Redondo-Gomez et al., 2006; Shabala et al., 2013), this result suggests that there was no damaged to PSII photochemistry due to applied levels of salinity and that the PSII system is not a main target of salinity stress. However, in more sensitive species such as faba bean 10 dS/m NaCl treatment has resulted in a significant decline in Fv/Fm values (Tavakkoli et al., 2010). A similar decline was also observed in *M. arborea* at higher irrigation salinities (200-300 mM; Boughalleb et al., 2009). Thus, there is some apparent threshold in the extent PSII can be protected against the detrimental effects of salinity.

The shoot biomass may also be influenced by the level of chlorophyll pigments in the plant leaf, as the amount of sequestered carbon (hence, yield) is generally proportional to the overall pigment content in the shoot (Ramesh et al., 2002; Islam et al., 2014). This statement can be supported by our observation where leaves with high chlorophyll content had high biomass yield in *M. arborea* (Fig. 1) as high chlorophyll content resulted in high photosynthesis rate. However, effects of salinity of pigment composition and chlorophyll content are not straightforward. While elevated cytosolic Na^+ and Cl^- may result in both reduce rate of chlorophyll biosynthesis and its faster degradation (Tavakkoli et al., 2010; Flowers et al., 2015), salinity also results in the smaller cell size and hence more dense pigment "packing". So, ultimately it is the total shoot chlorophyll content and the number of functional PSII units that matters for leaf photochemistry.

Impacts of soil texture on plant growth

Our results show that *A. lentiformis* and *M. arborea* grown in clay textured soil had higher biomass compared with the other soil texture situations regardless of the salinity or irrigation treatments. One reason for this higher growth could be the higher nutrient status measured in the clay soil used compared to the pure sandy loam or the sandy loam over the clay. What this study also shows is the low vs high irrigation rates had less impact than either the texture or salinity levels. This suggests waterlogging was not a significant issue. In *M. arborea,* which is a moderately salt-tolerant plant, a decline in biomass was expected in the high salinity situation but not in the same pattern in all soil texture set-ups. The physical properties of the soils, both soil texture and structure, may have played a role in the plant's performance because these soil properties affect the soil's nutrient holding capacity, aeration, pore space water-holding capacity and drainage (Warrence et al., 2002; Brady and Weil, 2009). Sandy soils generally have high infiltration rates and lower nutrient and water-holding capacity (Brady and Weil, 2009) whereas clay soils can hold more water but generally have slower infiltration and drainage rates. We suggest that in the sandy texture the applied water drained out more rapidly than the clay or the sandy loam/clay situation. Thus sandier soils would generally be preferable for high saline water irrigation (Warrence et al., 2002). However the high volumes of water and their salt content applied in this study may have leached more key plant nutrients out of the sandy loam than the clay textured media. This was shown in our field trials under high salinity irrigation (data not shown). This might explain the lower biomass yields of both species observed in the sandy loam despite this soil being more "immune" to salinity built up.

In general, in both species, clay-soil-grown plants had higher chlorophyll content and stomatal conductance compared to either the sandy loam over clay or the sandy loam soil in low irrigation (Fig. 3 and 4). We argued that soil structure and nutrient holding capacity may have been playing a role in this effect. Plants may have benefitted by the naturally high nutrient content of the clay soil (Table 1). In addition to this, it was also noted that in 16 dS/m saline treatment, lower leaf Na^+ concentration was obtained from clay soil compared to other soil types in both plant species. It could be possible that some of the Na^+ in the salt applied was absorbed in the soil particles and became less available for the plant for take up (e.g. shifting the ratio between exchangeable and solution Na^+). It has been reported that soil texture is strongly correlated with a soils ability to adsorb or desorb chemical ions (Miller and

Donahue, 1995). Clays provide cation exchange sites in soils and consequently are also a source for excess exchangeable Na^+ leading to swelling and dispersion (Miller and Donahue, 1995). It is also well known that Na^+ will accumulate in clay soils as opposed to sandy soils because of their lower leaching fraction and higher soil surface area (Warrence et al., 2002). It was also observed that in high salinity conditions *M. arborea* grown on clay soil had less leaf Cl^- concentration than plants grown on other soil types but just the opposite result was found in *A. lentirofmis*. Clay soils are predominantly negatively charged and applied Cl^- (via NaCl) is likely to be repelled (Bohn et al., 1979) and become easily available or leached through the soil. In general, halophytes maintain turgor by accumulating high Cl^- concentrations of 340 to 475 mM in plant tissues. Conversely, in glycophytes it is regarded as minor component of their cell sap osmotic pressure and has concentration of 7 to 70 mM (White and Broadley, 2001). This could be one of the reasons for having higher Cl^- in *A. lentiformis* compared to *M. arborea* that were grown in clay soil at the same salinity levels.

Tissue tolerance maybe conferring salinity tolerance in A. lentiformis but not in M. arborea

In this study, the overall plant performance of *Atriplex lentiformis* was better than the *Medicago arborea* up to the 16 dS/m water salinity despite the fact that overall Na^+ concentration was 2 – 3 times lower in *M. arborea* than *A. lentiformis*. This suggests an efficient tissue tolerance mechanisms were operating in *A. lentiformis*. Higher Na^+ and Cl^- values in A. *lentiformis* can be described as a typical characteristic of halophytic plants where they were known to accumulate Na^+ and Cl^- concentrations >500 mM (Flowers, 1985) to maintain a positive turgor under saline conditions. Some halophytes such *Tecticornia* species may accumulate close to 2M Na^+ on a tissue water basis when grown under highly saline conditions (English and Colmer, 2013). Shoot tissue tolerance in halophytes may be conferred by several different mechanisms (Shabala and Mackay 2011; Shabala, 2013; Shabala et al., 2014; Flowers et al., 2015) and these include: efficient vacuolar sequestration in leaf mesophyll; Na^+ sequestration in photosynthetically inactive tissues (such as parenchyma cells in succulent plants); Na^+ sequestration in external structures such as epidermal bladder cells (EBC); and Na^+ excretion through salt glands or glandular trichomes. The role of each of these mechanisms may differ depending on the species and its habitat. As *Atriplex* species contain well-developed dense layer of EBC on both surfaces of the leaf (Shabala and Mackay 2011; Shabala et al., 2014), it is plausible to suggest that external Na^+

sequestration may be the major contributor to the tissue tolerance in this species. It also suggested that lower Na leaf content in *Atriplex halimus* under high salinity condition may be related to the excretion of salt via vesiculated hairs present on the leaf (Belkheiri and Mulas 2013b). It was showed earlier for a related *Chenopodium quinoa* species (Bonales et al., 2013) that salt tolerance in young quinoa leaves possessing dense salt bladders was conferred predominantly by preferential Na^+ loading in EBC, while in old leaves where EBC were less numerous and not functional vacuolar Na^+ sequestration in leaf mesophyll was playing the critical role. In both cases, plants were efficient at removing the excessive Na^+ away from metabolically active cells of the growing plant body and minimised the effect of salinity (Shabala et al., 2014). We believe a similar scenario may be applicable for *Atriplex* species as well.

In the present study high level of Na^+ and K^+ ions in *A. lentiformis* observed in control plants (0.04 dS/m) indicates that these ions were used to maintain osmotic adjustment and probably plants accumulated those ions from the existing soil without supplemented by irrigation waters. Consistent with this notion, *A. lentiformis* had much higher Na^+ concentrations at low irrigation salinities compared with *M. arborea* but did not increase uptake much when concentration of salt was increased in irrigation water. This was not the case for *M. arborea*. Hence, control of the xylem Na^+ loading and its transport to the shoot has also contributed to differential growth patterns of species under saline conditions. The kinetics of this process warrants a separate investigation.

The ability of halophytes to sequester Na^+ in vacuoles is essential to adjust osmotic pressure and maintain positive shoot turgor and enable growth of new tissue (Glen et al., 1999; Flowers and Colmer, 2008). At the molecular level, the ability of halophytic plants to sequester huge amount of Na^+ in their vacuoles is related to the expression of tonoplast Na^+/H^+ antiporters under saline conditions (Barkla et al., 1995; Glenn et al., 1999, Apse and Blumwald, 2007). Importantly however, the activity of this exchanger has to be energized by either tonoplast H^+-ATPases (Vera-Estrella et al., 1999; Wang et al., 2001) or H^{+-} PP-ases (Krebs et al., 2010). Shabala (2013) suggested that overexpressing Na^+/H^+ NHX exchangers can only be fully functional under saline conditions if plants do not heavily invest available ATP pool for the production of compatible solutes (which otherwise used for fuelling tonoplast H^+-ATPase) and possess efficient K^+ retention mechanism (to enable tonoplast H^+-PPases to function). These assumptions seem to be met by *A. lentiformis* but not by *M*

arborea. Indeed, *A. lentiformis* had a high tissue K^+ concentration compared to *M arborea* in salinity treatments and was, therefore, more capable of maintaining (K^+-dependent; Rea and Poole, 1993; Shabala, 2013) operation of the tonoplast H^+-PPase. On the other hand, *M. arborea* grown at 16 dS/m salinity had higher Na^+ accumulation rate and low K^+ concentration. Based on the above assumption, it can be suggested that due to lack of sufficient K^+ (and, hence a failure to energize the tonoplast H^+-PPases) *M. arborea* may not have an ability to compartmentalise Na^+ within tissues by intracellular storage. So these accumulated high concentrations of Na^+ (in the metabolically active areas in the leaves) may have damaged the photosynthesis apparatus in *M. arborea.* As a result photosynthesis process reduced and growth is stunted.

5. Conclusion

Our results show that plant performance of both *Medicago arborea* and *Atriplex lenformis* was better in the clay texture compared to other soil texture setups under all irrigation treatments. There was no significant variation in chlorophyll fluorescence with salt treatments in both plant species but stomatal conductance was reduced in *M. arborea* when the level of salinity increased in the irrigation water. Overall, leaf ion content (Na^+ and Cl^-) also increased with increasing salinity treatment in both plants, but remarkable effects were seen only in sandy loam soil. This indicates that the soil texture and structure may have a significant role in the salt stress process under saline irrigation.

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Figure legends

Fig. 1. Effects of irrigation water salinity (0.04, 8, 16 dS/m) on mean ± S.E. fresh weight of leaf of *Atriplex lentiformis* (A, B*)* and *Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between soil treatments. (Low irrigation = 340 ml/pot/day, High irrigation = 680 ml/pot/day).

Fig. 2. Effects of irrigation water salinity (0.04, 8, 16 dS/m) on mean ± S.E. dry weight of leaf of *Atriplex lentiformis* (A, B) and *Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between soil treatments. (Low irrigation = 340 ml/pot/day, High irrigation = 680 ml/pot/day).

Fig. 3. Changes in stomatal conductance (gs) in *Atriplex lentiformis* (A, B) and *Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between soil treatments. (Low irrigation = 700 mm/year, High irrigation= 1300 mm/year).

Fig. 4. Mean SPAD reading in *Atriplex lentiformis* (A, B) and *Medicago arborea* (C, D*)*. Different letters above bars represent significant ($p<0.05$) difference between soil treatments. Data is mean ± s.e. (n = 5). (Low irrigation = 340 ml/pot/day, High irrigation= 680 ml/pot/day).

Fig. 5. Mean leaf sodium ion content in *Atriplex lentiformis* (A, B) and *Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between salinity treatments. Data is mean ± s.e. (n = 5). (Low irrigation = 340 ml/pot/day, High irrigation= 680 ml/pot/day).

Fig. 6. Leaf K^+ content in *Atriplex lentiformis* (A,B) *and Medicago arborea* (C,D*)*. Different letters above bars represent significant ($p<0.05$) difference between salinity treatment. Data is mean ± s.e. (n = 5). (Low irrigation = 340 ml/pot/day, High irrigation= 680 ml/pot/day).

Fig. 7. Leaf Cl^- content in *Atriplex lentiformis* (A,B) and *Medicago arborea* (C, D*)*. Different letters above bars represent significant ($p<0.05$) difference between salinity treatment. Data is mean ± s.e. (n = 5). (Low irrigation = 340 ml/pot/day, High irrigation= 680 ml/pot/day).

Fig. 8. Shoot sap osmolality (Osm; $mmolkg^{-1}$) of *Atriplex lentiformis* (A, B) *and Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between salinity treatment. Data is mean ± s.e. (n = 5). (Low irrigation = 340 ml/pot/day, High irrigation= 680 ml/pot/day).

Table.1 Initial selected soil chemical and nutritional properties of the soil used for the experiments. Clay soil used for this treatment was reactive clay from dolerite colluvium on the university farm and sandy loam was obtained from Males Sand Pit, South Arm Road, Tasmania.

Particulars	Clay soil	Sandy loam soil
Electrical conductivity saturated paste extract (dS/m)	1.02	0.41
pH (soil:water 1:5 ratio)	6.5	5.6
Total organic carbon (%)	1.98	1.36
Nitrate nitrogen (mg/kg)	95	23
Phosphorus Colwell (mg/kg)	36	17
Potassium Colwell (mg/kg)	230	98
Exchangeable ions (cmol(+))/kg		
Ca^{2+}	8.67	6.34
Mg^{2+}	3.21	2.23
Na^{+}	1.10	0.51
K^{+}	0.44	0.20

Table 2. Correlation matrix (Pearson's r values) calculated for a range of physiological characteristic me measured in *Atriplex lentiformis* (A) and M*edicago arborea* (B) grown in different level of salinity condition.

A						
	OSM	Leaf Na^+	Leaf K^+	Leaf Cl^-	LFW	SFW
OSM	n/a					
Leaf Na^+	0.80**	n/a				
Leaf K^+	-0.20	-0.16	n/a			
Leaf Cl^-	0.62**	0.54**	-0.18	n/a		
LFW	0.44**	0.41**	-0.28*	0.65**	n/a	
SFW	0.18	0.21	-0.04	0.45	0.81	n/a
B						
	OSM	Leaf Na^+	Leaf K^+	Leaf Cl^-	LFW	SFW
OSM	n/a					
Leaf Na^+	0.80**	n/a				
Leaf K^+	-0.22	-0.55**	n/a			
Leaf Cl^-	0.70**	0.76**	-0.32**	n/a		
LFW	-0.45**	-0.49**	0.04	-0.44**	n/a	
SFW	-0.53**	-0.52**	0.08	-0.45**	0.88	n/a

*Correlation is significant at 0.05 level; ** Correlation is significants at 0.01 level. OSM, Osmolality of leaf sap; leaf Na; Leaf Na concentration; Leaf K , Leaf K concentration, leaf Cl, leaf Cl^- concentration; LFW, leaf fresh weight; SFW, Stem Fresh weight.